FORM PTO-1390 (Modified) (REV 10-95) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR
194070US0PCT	09/600180

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/00635	01 FEBRUARY 1999	05 FEBRUARY 1998

TITLE OF INVENTION

APPARATUS FOR SYNTHESIS OF SUPPORT POLYMER MATERIALS IN THE FORM OF POROUS POLYMER BEADS

APPLICANT(S) FOR DO/EO/US

Christian MEIER, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

Items 13 to 18 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
    A **SECOND** or **SUBSEQUENT** preliminary amendment.
16. ☐ A substitute specification.
17. ☐ A change of power of attorney and/or address letter.
18. ☐ Certificate of Mailing by Express Mail
19. ☒ Other items or information:

Request for Consideration of Documents Cited in International Search Report
Notice of Priority
PCT/IB/308

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/600180	PCT/EP99/00635	194070US0PCT

20. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☒	Search Report has been prepared by the EPO or JPO	**$840.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482)	**$670.00**
☐	No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))	**$760.00**
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO	**$970.00**
☐	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)	**$96.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =	**$840.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$130.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	10 - 20 =	0	x $18.00	**$0.00**	
Independent claims	1 - 3 =	0	x $78.00	**$0.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$970.00**	
Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**.			☐	**$0.00**	
SUBTOTAL =				**$970.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)).			+	**$0.00**	
TOTAL NATIONAL FEE =				**$970.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			☐	**$0.00**	
TOTAL FEES ENCLOSED =				**$970.00**	
				Amount to be: refunded	$
				charged	$

☒ A check in the amount of **$970.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **15-0030** A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Surinder Sachar
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Surinder Sachar
SIGNATURE

Norman F. Oblon
NAME

24,618
REGISTRATION NUMBER

Aug. 4, 2000
DATE

194070US0PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :

CHRISTIAN MEIER ET AL. :

SERIAL NO: NEW APPLICATION : ATTN: APPLICATION BRANCH
(BASED ON PCT/EP99/00635)

FILED: HEREWITH :

FOR: APPARATUS FOR SYNTHESIS OF SUPPORT POLYMER MATERIALS IN THE FORM OF POROUS POLYMER BEADS

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows.

IN THE SPECIFICATION

Please amend the specification as follows:

Page 1, before line 1, delete the title of the invention in its entirety, and insert therefor:

- - DEVICE FOR PRODUCING POLYMER SUPPORT MATERIALS IN THE FORM OF POROUS POLYMER BEADS - -.

IN THE CLAIMS

Please amend the claims as follows:

Claim 2, line 1, change "characterized in that" to --wherein--.

Claim 3, line 1, change "characterized in that" to --wherein--.

4. (Amended) A support polymer material which can be synthesized by a process according to Claim 1, wherein [one or more of claims 1 to 3, characterized in that] it has a binding capacity for penicillin amidase from *E. coli* of at least 220 [U/g moist], resulting from the reaction of 1530 units of penicillin amidase with 1 g of support polymer material, and exhibits a swelling factor of at most 1.5.

REMARKS

Claims 1-10 are active in the present application. The claims are amended for clarity. No new matter has been added. Applicants submit that the present application is now in condition for examination on the merits. Early notice of such is earnestly solicited.

Respectfully submitted,

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194070US0PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF: :

CHRISTIAN MEIER ET AL. :

SERIAL NO: NEW APPLICATION : ATTN: APPLICATION BRANCH
(BASED ON PCT/EP99/00635)

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FOR: APPARATUS FOR SYNTHESIS OF SUPPORT POLYMER MATERIALS IN THE FORM OF POROUS POLYMER BEADS

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Prior to examination on the merits, please amend the above-identified application as follows.

IN THE SPECIFICATION

Please amend the specification as follows:

Page 1, before line 1, delete the title of the invention in its entirety, and insert therefor:

--DEVICE FOR PRODUCING POLYMER SUPPORT MATERIALS IN THE FORM OF POROUS POLYMER BEADS--.

IN THE CLAIMS

Please amend the claims as follows:

Claim 2, line 1, change "characterized in that" to --wherein--.

Apparatus for synthesis of support polymer materials in the form of porous polymer beads

The invention relates to a process for synthesis, by inverse suspension polymerization of a monomer phase, of a bead-like, cross-linked, hydrophilic copolymer which has binding activity toward ligands containing nucleophilic groups. The invention also relates to support polymer materials with high binding capacity for penicillin amidase and low swelling factor, as well as to use of the same.

Prior art

Porous polymer support materials for proteins, especially enzymes, are sufficiently known. Applications exist in medicine, for example, in the enzyme-induced cleavage of β-lactam antibiotics such as penicillin G to 6-aminopenicillanic acid (6-APA) by means of penicillin acylase (penicillin amidase). Important development goals are primarily the highest possible loading capacity, low swelling ability and the lowest possible residual solvent contents. Halogenated solvents should in principle be avoided for synthesis.

German Laid-open Application DE-OS 2237316 describes a process for synthesis of bead-like, cross-linked copolymers by radical polymerization of a monomer mixture containing a radical-forming initiator and comprising a monomer having binding activity toward biological substances, a cross-linking monomer and at least one further comonomer, the said monomer mixture being suspended as droplets and polymerized in a nonpolar organic liquid. Aliphatic hydrocarbons in particular, and above all such with 8 and more C atoms, are suitable as the nonpolar organic liquid.

Mixtures of n-heptane and perchloroethylene are used in the examples. The ratio of the monomer phase to the organic dispersion medium can range between 1:1 and 1:10, but ratios of between 1:1.5 and 1:4 are preferred. German Patent DE A 3106456 describes a process improved compared with DE-OS 2237316 in relation to the binding capacity of the polymer beads. Particularly high binding capacities for proteins, especially for the penicillin acylase (penicillin amidase) enzyme are obtained when the support polymers contain high contents of cross-linking monomers and when the monomer phase, formed from the monomers and the diluent, contains a solvent mixture as diluent. Suitable mixtures can be, for example, water/methanol or formamide/methanol. Monomers and diluents are present in a ratio of about 1:2.6. A mixture of n-hexane and perchloroethylene is used as the organic, dispersion medium. In the examples, the ratio of the monomer phase to the organic dispersion medium is about 1:2.8. When the proportion of cross-linking agent in the monomer mixture is 50 wt% and water/methanol is used as the diluent, there can be obtained support polymers with a binding capacity of up to 125 U/g, measured as penicillin acylase activity.

Object and achievement

The object of the invention is to provide an improved process for synthesis of bead-like, cross-linked copolymers. It is also the intent to avoid the use of halogenated solvents in the organic dispersion medium and at the same time to achieve a binding capacity of at least 220 [U/g moist] for the penicillin amidase enzyme (EC 3.5.1.11) under standardized conditions (loading of 1 g of support polymer material with 1530

units of penicillin amidase). Furthermore, the swellability of the polymer beads in water should not exceed 1.5, expressed as a swelling factor (ml moist/ml dry).

The object was achieved by a process for synthesis, by inverse bead polymerization of a monomer phase, of a bead-like, cross-linked, hydrophilic copolymer which has binding activity toward ligands containing nucleophilic groups, which monomer phase comprises monomers and a diluent, which contains as monomers

a) 5 to 40 wt% of hydrophilic monomers which contain a vinyl group, can undergo radical polymerization and form at least 10% aqueous solutions at room temperature

b) 30 to 50 wt% of monomers which contain a vinyl group and an additional functional group, can undergo radical polymerization and, in a polymer-like reaction with the nucleophilic groups of the ligands, can form covalent bonds

c) 20 to 60 wt% of hydrophilic, cross-linking monomers which contain two or more ethylene-type unsaturated polymerizable groups and can undergo radical polymerization,

with the proviso that a), b) and c) add up to 100 wt%, which uses as diluent a mixture of methanol and water in the ratio of 1:1.0 to 1:4.0, the monomer phase being dispersed as droplets in a dispersion medium comprising an organic solvent chosen from the aliphatic hydrocarbons with 5 to 7 carbon atoms, the ratio of monomer

phase to dispersion medium ranging from 1:2.0 to 1:4.0, and which in this form is subjected to radical polymerization in the presence of a polymerization initiator and a protective colloid, with the proviso that the ratio of monomers to diluent ranges from 1:1.7 to 1:2.4.

By application of the inventive process it is possible to obtain a novel support polymer material, which has a loading capacity for penicillin amidase of at least 220 [U/g moist], resulting from the reaction of 1530 units of penicillin acylase with 1 g of support polymer material, and which exhibits a swelling factor of at most 1.5.

It was not foreseeable that the definition of the various process parameters relative to each other would lead to a clearly greater binding capacity for the penicillin amidase enzyme and that at the same time, however, the swellability would decrease. It was also surprising that, by application of the inventive process, the use of halogenated hydrocarbons such as perchloroethylene, which heretofore have been the most widely used compounds for equalizing the densities of the phases, can be avoided by choosing as the organic solvent an aliphatic hydrocarbon with 5 to 7 carbon atoms.

Operation of the invention

Monomers

In order to ensure that the monomer mixture is hydrophilic, it must comprise predominantly hydrophilic monomers. As hydrophilic monomers there are to be understood such monomers that form at least 10% aqueous solutions at room temperature and preferably do not contain any ionic groups or groups that can be ionized by addition of acids or bases.

Monomers a) comprise 5 to 40 wt%, 8 to 35 wt%, especially 9 to 12 wt% of hydrophilic monomers which contain a vinyl group, can undergo radical polymerization and form at least 10% aqueous solutions at room temperature.

Suitable as monomers a) are in particular acrylamide and/or methacrylamide, but methacrylamide is preferred. Further examples are hydroxyalkyl esters of unsaturated polymerizable carboxylic acids, such as hydroxyethyl acrylate and hydroxyethyl methacrylate or N-vinylpyrrolidone.

Monomers b) comprise 30 to 50 wt%, preferably 35 to 45 wt% of monomers which contain a vinyl group and an additional functional group, preferably an oxirane group (epoxy group), can undergo radical polymerization and, in a reaction analogous to polymerization, can form covalent bonds with the nucleophilic groups of the ligands. Oxirane groups in particular are suitable for binding ligands while preserving their biological activity.

Preferred monomers b) are glycidyl methacrylate and/or allyl glycidyl ether. Especially preferably, both monomers are used in approximately equal proportions at the same time.

Monomers c) comprise 20 to 60 wt%, especially 25 to 55 wt%, especially preferably 40 to 55 wt% of hydrophilic, cross-linking monomers which contain two or more ethylene-type unsaturated polymerizable groups and can undergo radical polymerization. Preferred monomers c) are N,N'-methylenebisacrylamide or N,N'-methylenebismethacrylamide. N,N'-Methylenebismethacrylamide is especially preferred. If necessary, 0 to 10 wt% of further cross-linking monomers which contain two or more ethylene-type unsaturated polymerizable groups and can undergo radical polymerization may also be used. Suitable are hydrophilic di(meth)acrylates such as polyethylene oxide di(meth)acrylates.

Monomers a), b) and c) add up to 100 wt% in all cases.

Diluent

The monomer phase comprises monomers a) to c), which are dissolved in a diluent, which must be a mixture of methanol and water in the ratio 1:1.0 to 1:4.0. Especially favorable mixing ratios for methanol and water range from 1:1.2 to 1:2.5, especially from 1:1.3 to 1:1.7.

Ratio of monomers to diluent

The ratio of monomers to diluent is especially critical. It must range from 1:1.7 to 1:2.4, especially preferably from 1.9 to 2.1.

Dispersion medium

An organic solvent comprising an aliphatic hydrocarbon with 4 to 7 C atoms is suitable as the dispersion medium. n-Heptane is preferred and cyclohexane is especially preferred.

Ratio of monomer phase to dispersion medium

The ratio of the monomer phase to the dispersion medium formed by the organic solvent must range from 1:2.0 to 1:4.0, preferably from 1:2.8 to 1:3.3.

Further process conditions

As further constituents the suspended monomer phase contains polymerization initiators which are known in themselves, preferably sulfur-free initiators and especially preferably 4,4'-azobis-(4-valeric acid), as well as protective colloids (emulsifiers), such as a copolymer comprising 95 parts of n-butyl methacrylate and 5 parts of 2-trimethylammoniumethyl methacrylate chloride with molecular weights (weight-average) in the range of 30,000 to 80,000.

The bead polymerization (also known as suspension polymerization) is otherwise

performed in known manner, for example by firstly introducing the dispersion medium and the protective colloid, then dispersing the monomer phase, which also contains the initiator, in the organic phase with stirring at 40 to 60°C, for example, and then heating to 60 to 70°C. The water/methanol mixture can be removed from the loop almost completely in the form of an azeotrope over a period of, for example, 6 hours. The mixture is allowed to react to completion for about 3 to 5 hours and is then cooled to room temperature. The resulting beads are suctioned and dried in vacuum for a period of, for example, 12 hours. Alternatively, the bead polymers can also be filtered off and washed with water. Drying is preferably performed in a fluidized-bed dryer, since in this way solvent residues can be removed particularly effectively.
The obtained polymer beads (= support polymer material) have a size in the range of 50 to 500 μm, especially of 120 to 250 μm.
By binding capacity there is understood that enzyme activity which can be achieved when the support polymer material is loaded to the maximum with a specified enzyme. An important application of the inventive support polymer material is the cleavage of penicillin G to 6-aminopenicillanic acid (6-APA) by means of bound penicillin amidase from *E. coli*. The binding capacity is expressed as penicillin amidase activity in units per g of support polymer beads [U/g moist]. The binding capacity of the inventive support polymer beads in this measurement method is at least 220 [U/g moist].

The swellability of the polymer beads in water is expressed by the swelling factor [ml moist/ml dry]. The inventive polymer beads exhibit a swelling factor of no greater than 1.5.

Uses of the inventive support polymer materials

The inventive support polymer materials can be used in stirred or flow reactors for covalent binding of ligands by means of the oxirane groups which they contain. This can be achieved, for example, by addition of proteins, especially enzymes, from concentrated solutions via covalent bonding with retention of their biological activity. Peptides, amino acids, β-lactam antibiotics, lipids, nucleotides, polynucleotides, low molecular weight nucleophilic compounds or metalloorganic compounds can also be reacted with the oxirane groups of the support beads.

The polymer beads loaded with ligands can be used in procedures known in themselves for stereospecific synthesis of chiral substances such as amino acids (d-phenylalanine, p-hydroxy-d-phenylalanine, l-tert-leucine) or of pharmaceuticals such as ibuprofen. They are also used as supports in enzyme-induced cleavage of penicillin G to 6-aminopenicillanic acid (6-APA), of cephalosporin G to 7-aminodesacetoxycephalosporanic acid (7-ADCA) or of cephalosporin C to 7-aminocephalosporanic acid (7-ACA). The process is described in DECHEMA Annual Conference 1996 - Abstracts [in German], Vol. 1, DECHEMA e.V. Further applications are specific enzyme-induced syntheses of amoxicillin and ampicillin on substrates such as the above cleavage products. A further application comprises syntheses of fine chemicals or basic products (such as malic acid) for chemical syntheses.
The polymer beads can also be used in separation technology for adsorption chromatography or gel permeation chromatography. To achieve specific adsorption, the polymer beads can be loaded with immunoglobulin fractions from antiserums or

with monoclonal antibodies. The use of support polymer material loaded with enzymes or antibodies as adsorbent in extracorporeal therapy, in which pathogenic or toxic substances are removed from whole blood, can be cited as yet a further application.

Examples

(The determination method hereinafter is familiar in itself to the person skilled in the art of support polymer materials, and will be described only for the sake of completeness)

Determination of the binding capacity for penicillin amidase (= penicillin G acylase) from *E. coli* (EC 3.5.1.11)

a) Covalent binding of penicillin amidase to the support polymer material

1 g of support polymer material was added to 1530 units of penicillin amidase in 5 ml of sterile 1 M potassium phosphate buffer of pH 7.5 and incubated for 48 hours at 23°C.

Thereafter the polymer beads were placed on a sintered glass filter (porosity 2 or 3) and, in a suction process, washed on the filter two times with deionized water and then two times with 0.1 M potassium phosphate buffer of pH 7.5 containing 0.05% ethyl-4-hydroxybenzoate. The moist weight of the resulting beads loaded with penicillin acylase was determined.

b) Determination of the binding capacity

250 to 300 mg of moist support polymer material (polymer beads) coupled with

penicillin amidase was added to 20 ml of a 2% penicillin G solution in 0.05 M potassium phosphate buffer of pH 7.5, containing 0.05% ethyl-4-hydroxybenzoate. and maintained at 37°C. Liberated phenylacetic acid was titrated under steady stirring with 0.5 M NaOH at a constant pH of 7.8 for a period of 10 minutes, during which the NaOH consumption was recorded.

Thereafter the polymer beads were collected as under a) on a sintered glass filter by means of suctioning of 20 ml of 0.05 M potassium phosphate buffer of pH 7.5 containing 0.05% ethyl-4-hydroxybenzoate, and the measurement was repeated two times.

c) Calculation of the binding capacity

The linear region of the measured curve (usually the region from 1 to 5 minutes) was used as basis for the calculation and extrapolated to an interval of 10 minutes. The binding capacity was expressed as units of penicillin amidase per g of moist support polymer material (U/g moist). One unit corresponds to one μmol of hydrolyzed penicillin G per minute (μmol/min); thus 1 liter of 0.5 M NaOH is equivalent to 500 μmol of hydrolyzed penicillin G. (The water content of the support polymer material is approximately constant and can therefore be disregarded.)

Examples 1 to 3

Test conditions common to Examples 1 to 3:

In a 2-liter stirred flask with thermometer, water separator, reflux condenser and nitrogen admission tube there were placed an organic solvent, 3 g of a copolymer comprising 95 parts of n-butyl methacrylate and 5 parts of 2-trimethylammoniumethyl methacrylate chloride as protective colloid and 5 g of dry ice. Under stirring and passage of nitrogen, there was dispersed in the organic phase at 50°C a monomer phase comprising water and methanol in a ratio of 1:1.5 as diluent, plus

10 g of methacrylamide,
20 g of allyl glycidyl ether,
20 g of glycidyl methacrylate and
50 g of methylenebismethacrylamide

plus

2 g of 4,4'-azobis-4-cyanovaleric acid (as polymerization initiator),

after which the contents were heated to boiling at 65 to 70°C. The mixture was incubated for about 6 hours and then cooled to room temperature. The resulting polymer beads were suctioned, washed and dried in the fluidized-bed dryer. Thereafter the binding capacity for penicillin amidase [U/g moist] and the swelling factor [ml moist/ml dry] were determined.

The main test parameters and the results of Examples 1 to 3 are presented in the following table.

	Example 1 (according to the invention)	Example 2 (comparison example)	Example 3 (comparison example)
Organic solvent (dispersion medium)	952 g of cyclohexane	669 g of cyclohexane	530 g of n-heptane + 530 g of perchloroethylene
Total monomers	100 g	100 g	100 g
Diluent	80 g of methanol + 120 g of water (= 1:1.5)	263 g of formamide	264 g of formamide
Monomers + diluent (monomer phase)	300 g	363 g	364 g
Ratio of monomer to diluent	1:2	1:2.63	1:2.64
Ratio of monomer phase to dispersion medium	1:3.2	1:1.8	1:2.9
Binding capacity for penicillin amidase (1530 U) [U/g moist]	252	194	192
Swelling factor [ml moist/ml dry]	1.3	4.0	3.9

CLAIMS

1. A process for synthesis, by inverse bead polymerization of a monomer phase, of a bead-like, cross-linked, hydrophilic copolymer which has binding activity toward ligands containing nucleophilic groups, which monomer phase comprises monomers and a diluent, which contains as monomers

a) 5 to 40 wt% of hydrophilic monomers which contain a vinyl group, can undergo radical polymerization and form at least 10% aqueous solutions at room temperature

b) 30 to 50 wt% of monomers which contain a vinyl group and an additional functional group, can undergo radical polymerization and, in a polymer-like reaction with the nucleophilic groups of the ligands, can form covalent bonds

c) 20 to 60 wt% of cross-linking monomers which contain two or more ethylene-type unsaturated polymerizable groups and can undergo radical polymerization,

with the proviso that a), b) and c) add up to 100 wt%, which uses as diluent a mixture of methanol and water in the ratio of 1:1.0 to 1:4.0, the monomer phase being dispersed as droplets in a dispersion medium comprising an organic solvent chosen from the aliphatic hydrocarbons with 5 to 7 carbon atoms, the ratio of monomer phase to dispersion medium ranging from 1:2.0 to 1:4.0, and which in this form is

subjected to radical polymerization in the presence of a polymerization initiator and a protective colloid, with the proviso that the ratio of monomers to diluent ranges from 1:1.7 to 1:2.4.

2. A process according to claim 1, characterized in that there are used as monomers

a) acrylamide and/or methacrylamide

b) glycidyl methacrylate and/or allyl glycidyl ether

c) methylenebisacrylamide or methylenebismethacrylamide.

3. A process according to claim 1, characterized in that cyclohexane is used as the organic solvent.

4. A support polymer material which can be synthesized by a process according to one or more of claims 1 to 3, characterized in that it has a binding capacity for penicillin amidase from *E. coli* of at least 220 [U/g moist], resulting from the reaction of 1530 units of penicillin amidase with 1 g of support polymer material, and exhibits a swelling factor of at most 1.5.

5. The use of the support polymer material according to claim 4 for binding of proteins.

6. The use of the support polymer material according to claim 5 for binding of enzymes.

7. The use of the support polymer material according to claim 5 for binding of antibodies.

8. The use of the support polymer material according to claim 4 in chromatography.

9. The use of the support polymer material according to claim 4 for synthesis of pharmaceuticals.

10. The use of the support polymer material according to claim 4 for stereospecific synthesis of chiral substances.

ABSTRACT

The invention relates to a process for synthesis, by inverse bead polymerization of a monomer phase, of a bead-like, cross-linked, hydrophilic copolymer which has binding activity toward ligands containing nucleophilic groups. The invention relates to support polymer materials with high binding capacity for penicillin acylase and low swelling factor, as well as to use of the same.

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Declaration and Power of Attorney for Patent Application
Erklärung für Patentanmeldungen mit Vollmacht
German Language Declaration

Als nachstehend benannter Erfinder erkläre ich hiermit an Eides Statt:

daß mein Wohnsitz, meine Postanschrift und meine Staatsangehörigkeit den im nachstehenden nach meinem Namen aufgeführten Angaben entsprechen, daß ich nach bestem Wissen der ursprüngliche, erste und alleinige Erfinder (falls nachstehend nur ein Name angegeben ist) oder ein ursprünglicher, erster und Miterfinder (falls nachstehend mehrere Namen aufgeführt sind) des Gegenstandes bin, für den dieser Antrag gestellt wird und für den ein Patent für die Erfindung mit folgendem Titel beantragt wird:

deren Beschreibung:

☐ ist beigefügt

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unter der US-Anmeldenummer oder unter der Internationalen Anmeldenummer im Rahmen des Vertratgs über die Zusammenarbeit auf dem Gebiet des Patentwesens (PCT)

_____ und am

_____ abgeändert (falls zutreffend).

Ich bestätige hiermit, daß ich den Inhalt der oben angegebenen Patentanmeldung, einschließlich der Ansprüche, die eventuell durch einen oben erwähnten Zusatzantrag abgeändert wurde, durchgesehen und verstanden habe.

Ich erkenne meine Pflicht zur Offenbarung jeglicher Informationen an, die zur Prüfung der Patentfähigkeit in Einklang mit Titel 37, Code of Federal Regulations, § 1.56 von Belang sind.

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

DEVICE FOR PRODUCING POLYMER SUPPORT MATERIALS IN THE FORM OF POROUS POLYMER BEADS (as amended)

the specification of which:

☐ is attached hereto.

☒ was filed on August 4, 2000

as United States Application Number or PCT International Application Number

09/600,180 and was amended on

_____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

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Ich beanspruche hiermit ausländische Prioritätsvorteile gemäß Title 35, US-Code, § 119(a)-(d), bzw. § 365(b) aller unten aufgeführten Auslandsanmeldungen für Patente oder Erfinderurkunden, oder § 365(a) aller PCT internationalen Anmeldungen, welche wenigstens ein Land ausser den Vereinigten Staaten von Amerika benennen, und habe nachstehend durch ankreuzen sämtliche Auslandsanmeldungen für Patente bzw. Erfinderurkunden oder PCT internationale Anmeldungen angegeben, deren Anmeldetag dem der Anmeldung, für welche Priorität beansprucht wird, vorangeht.

I hereby claim foreign priority under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below, and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior foreign application(s)
(Frühere ausländische Anmeldungen)

Priority claimed
Priorität beansprucht

(Number) (Nummer)	(Country) (Land)	(Day/Month/Year Filed) (Tag/Monat/Jahr der Anmeldung)	Yes Ja	No Nein
198 04 518.2	GERMANY	05 February 1998	☒	☐
			☐	☐

Ich Beanspruche hiermit Prioritätsvorteile unter Title 35, US-Code, § 119(e) aller US-Hilfsanmeldungen wie unten aufgezählt.

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(Application No.) (Aktenzeichen)	(Filing Date) (Anmeldetag)	(Application No.) (Aktenzeichen)	(Filing Date) (Anmeldetag)

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(Application No.) (Aktenzeichen)	(Filing Date) (Anmeldetag)	(Status) (patented, pending, abandoned) (Status) (patentiert, schwebend, aufgegeben)
PCT/EP99/00635	01 February 1999	

Ich erkläre hiermit, daß alle in der vorliegenden Erklärung von mir gemachten Angaben nach bestem Wissen und Gewissen der Wahrheit entsprechen, und ferner daß ich diese eidesstattliche Erklärung in Kenntnis dessen ablege, daß wissentlich und vorsätzlich falsche Angaben oder dergleichen gemäß § 1001, Title 18 des US-Code strafbar sind und mit Geldstrafe und/oder Gefängnis bestraft werden können und daß derartige wissentlich und vorsätzlich falsche Angaben die Rechtswirksamkeit der vorliegenden Patentanmeldung oder eines aufgrund deren erteilten Patentes gefährden können.

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POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: *(list name and registration number)*

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